Calibration (The Good Curve)

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Abstract

- It should come as no surprise that the correlation coefficient is only one way to judge the quality of a calibration curve.
- The fact is that there are numerous tools available to evaluate how good a calibration curve represents a given data set.
- With a basic understanding of how to use regression analysis, some simple methods can be applied to determine both the quality of necessary to improve calibration technique.

Overview

- The black box versus thinking outside the box.
- The black box is the regression algorithm.
- Thinking outside the box is evaluating analytical processes or biases which may effect the quality of the curve.

What is a Curve?

- *STANDARD CURVE: A plot of concentrations of known analyte standards versus the instrument response to the analyte. Calibration standards are prepared by successively diluting a standard solution to produce working standards which cover the working range of the instrument. Standards should be prepared at the frequency specified in the appropriate section....* *SW-846*

OK, what do we really mean by curve?

- The ability to predict behavior by establishing known outcome.
- Simply put, getting a result with reasonable certainty.

What' so important about a good Curve?

- Easier to correct problems if the calibration is bad
- Higher confidence in analytical results

A Peek inside the Black Box

- Basic linear regression by least squares
- LINEAR Eq.: x=(y-b)/m or y=mx+b
- where m = slope or rise over run
- b = intercept (curve crosses the y axis)
- $$\text{Slope} = \frac{(\Sigma xy - (\Sigma x * \Sigma y / n))}{(\Sigma x^2 - ((\Sigma x)^2 / n))}$$
- $$\text{intercept} = (\Sigma y/n) - (m * \Sigma x / n)$$

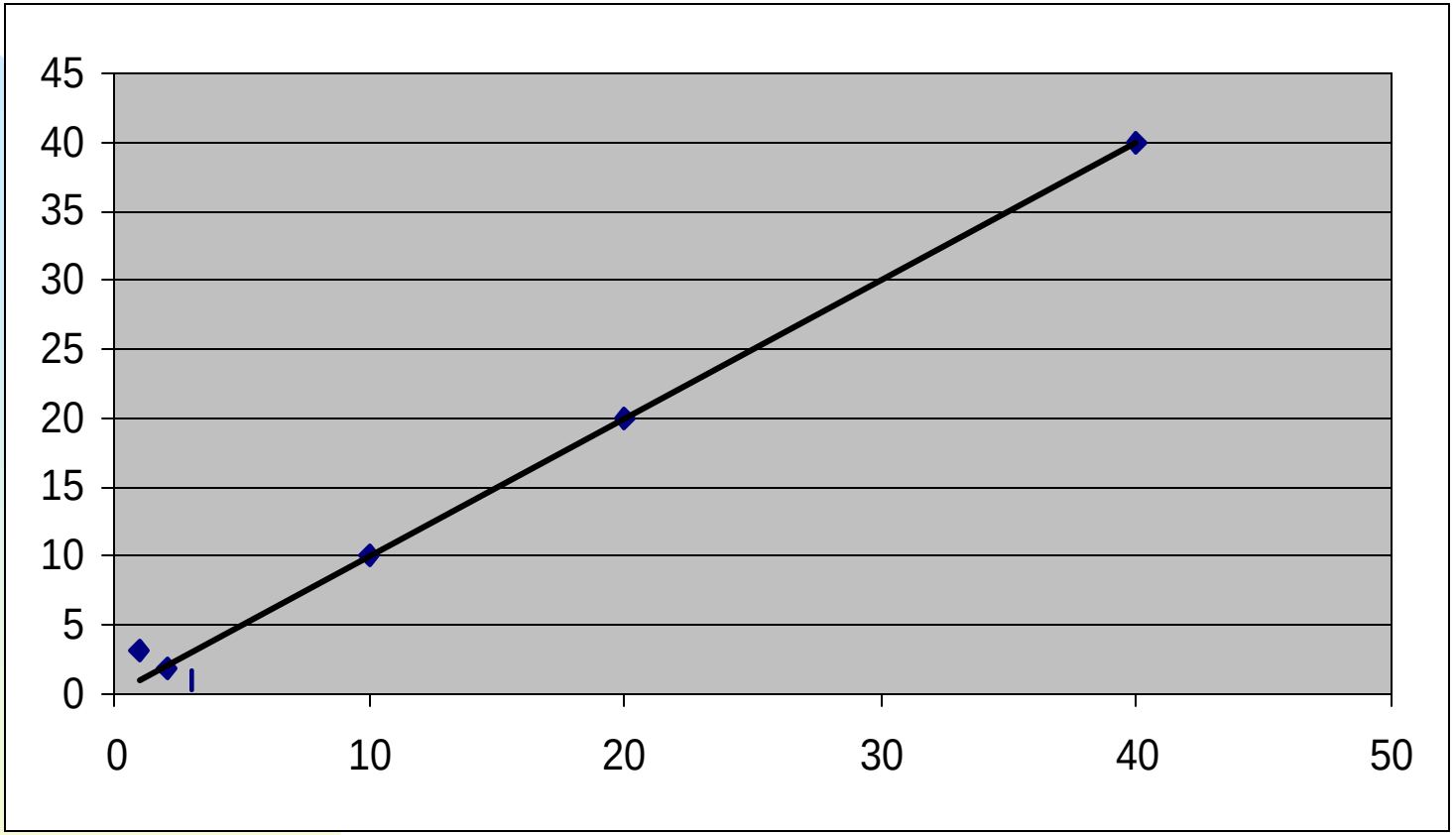
The Correlation Coefficient

- An estimate of the remainders derived from the regression.
  - How do we calculate it?

$$r = \frac{(\Sigma xy - (\Sigma x * \Sigma y / n))^2}{((\Sigma x^2 - ((\Sigma x)^2 / n)))*(\Sigma y^2 - ((\Sigma y)^2 / n))}$$

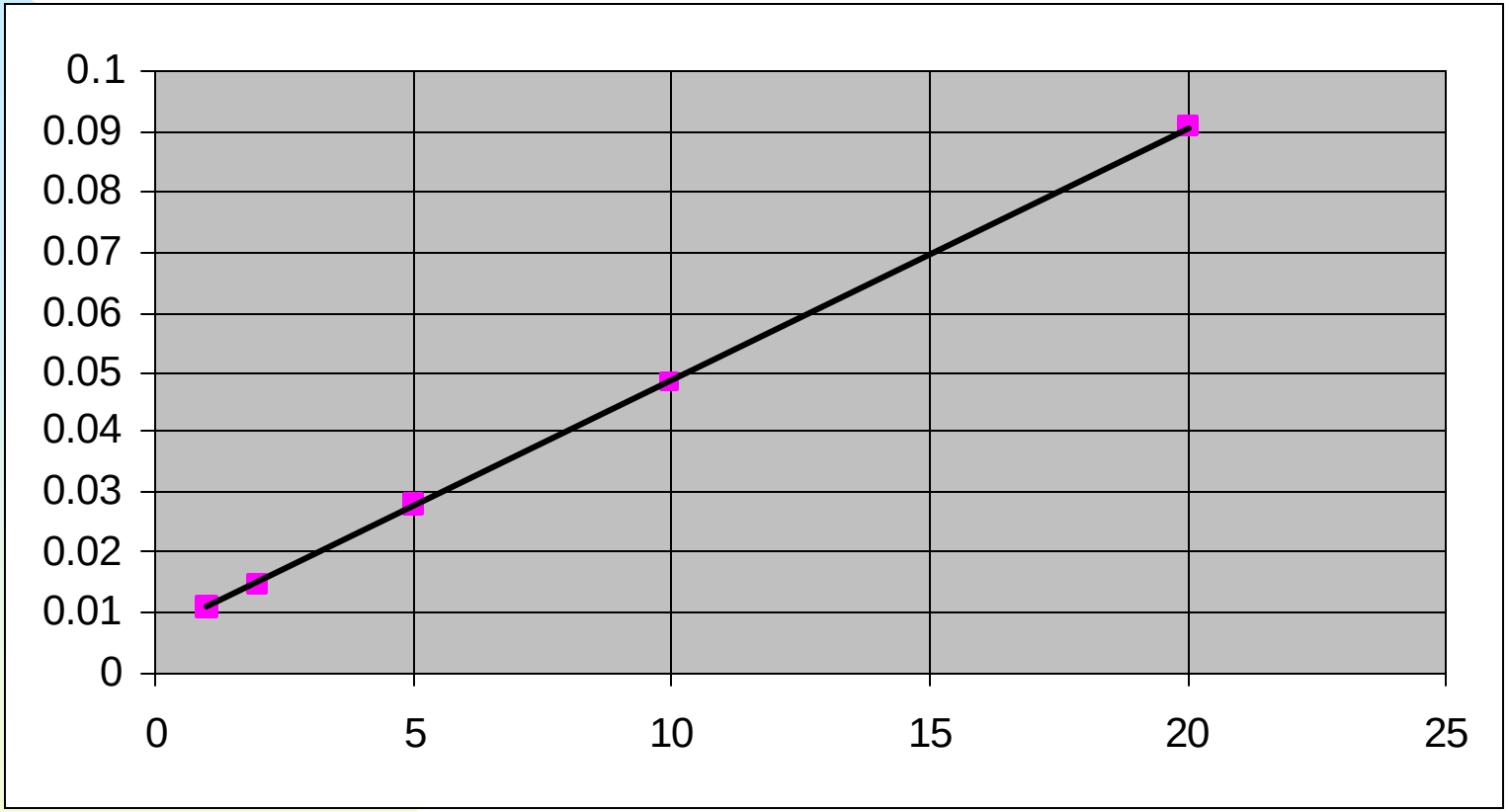
  - What does it mean?
  - For our purpose it must be >0.995
  - The correlation coefficient only indicates variance from the averages; therefore it is not the best indicator of curve quality.

r = 0.997



Std 1	3	1
Std 2	2	2
Std 3	1	3
Std 4	10	10
Std 5	20	20
Std 6	40	40

r = 0.9999



Std 1	1	0.011	90.90909
Std 2	2	0.015	133.3333
Std 3	5	0.028	178.5714
Std 4	10	0.048	208.3333
Std 5	20	0.091	219.7802

Outside the Box

- Beer's Law states that the absorbance of a solution is directly proportional to the concentration. (Ideally linear)

- What effects Beer?
  - Noise is most often the function of the detector
  - Quenching - At high concentration, Beer's law will not continue to behave
  - Limiting factors of method or reagents. Most methods are intended to operate at specific trace ranges. Dilution after reaction or extraction beyond trace levels may bias final results. Capture

Evaluation of Curve Quality

- Slope vs. RF
  - Response Factor is the ratio of Concentration/Response and represents the instantaneous slope for a given point.
- Variance of RF
  - The average of the RFs should be close to the slope. The variance of the RFs is an excellent indication of curve quality. RSD <10%
- Linear Calibration Range Study - LCR
  - LCR is simply plugging the response of each standard into the line function and determining the variance from predicted value (conc.). These should be <20% and ideally <10%
- Intercept - Should not be greater than the lowest standard.
- Visual
  - Date should appear to be linear and fall along the curve.
- Equal Data points on either side
  - Generally, data points should be evenly distributed around the curve. If most of the points fall to one side of the line or are not evenly divided by the line, there may be a problem with the curve.

Problems Effecting Curve Quality

- Bias
  - Bias is any part of the analytical process which may cause unequal treatment from one part of the procedure to another- such as using that "special" beaker for blanks.
- Intercept
  - High intercepts imply bias for instrument background or contamination.
- Tilt
  - Tilt or skewed sloped is often the result of poor instrument performance.
- Higher order curves
  - Calibration curves which are not linear may imply problems in the analytical process such as saturation or degradation.
- Bimodal data
  - Bimodal data may appear to have two distinct 'lines' and is most often related to dilution technique like using a eppendorf for the lower part of a curve and a pipet for the upper part of the curve.
- Scatter
  - Random divergence in a curve can result from numerous factors including extraction/digestion, poor technique, or dirty or malfunctioning instrumentation among others.

Problems Effecting Curve Quality

- Forcing through zero
  - Linear regression should not be forced through zero for two reasons:
  - 1) Good information can be gained from the natural intercept.
  - 2) The regression formula is skewed by passing through zero.
- Span
  - The entire range of the analytical curve can be problematic if it goes beyond the linear range of the instrument or system.
- Spacing of stds
  - Standards should represent the analytical range evenly. Keep in mind that the Least Squares algorithm can be biases by using higher concentrations. Standards should not exceed five (5) fold changes in concentration at most.
- Dilution and general pipetting technique
  - It is critical to use consistent and precise dilution techniques for standards and all spiking or QC functions. Use of varying means for dilutions can add significant variance for each standard.

Corrective Actions

- Perform Maintenance
- Clean glassware
- Check dilutions and be consistent
- Remake standard and reagents (INCLUDING STOCKS)
- Re-run standards
- Re-zero

- Avoid:
  - Deleting standards
  - Running extra standards
  - Excessive re-zeroing
  - "Special" Behavior (i.e. using one cuvette for standards and another for samples)

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Discussion/Questions

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